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Original Article

Pharmacokinetic variability of clarithromycin and differences in CYP3A4 activity in patients with cystic fibrosis☆

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Abstract

Background: To investigate the correlation between CYP3A4/5 activity and clarithromycin metabolism, and between CYP3A activity and *CYP3A* genotype.
Methods: This is an open-label, prospective pharmacokinetic study evaluating CYP3A activity using The Erythromycin Breath Test. Eight blood samples were collected within 12 h after clarithromycin 500 mg was administered orally. The clarithromycin concentrations were measured by liquid chromatography–tandem mass spectrometry. AUC, Tmax and Cmax were calculated. Selected Single Nucleotide polymorphisms in *CYP3A4/5* genes were assessed by PCR and single base extension.
Results: Twenty-one chronically infected patients were included. An 8-fold variation in the CYP3A4 activity, 10-fold variation in AUC for clarithromycin (median 881 μg/mL × min), and a 16-fold variation in Cmax for clarithromycin (median 3.4 μg/mL) were found. A linear correlation between the CYP3A4-activity and clarithromycin metabolism was demonstrated ($P < 0.05$).
Conclusion: The large variation in the clarithromycin pharmacokinetics in cystic fibrosis patients may cause treatment failure. The Erythromycin Breath Test could be valuable in identifying cystic fibrosis patients in risk of treatment failure/drug toxicity.

Keywords: SNPs; ERMBT; AUC; Cytochrome P450

1. Introduction

Chronic pulmonary infections increase the morbidity and mortality in patients with cystic fibrosis (CF). The introduction of antibiotic treatment with regular intervals has improved their survival significantly and children born today with CF have a predicted median survival of close to 40 years in USA [1] and more than 50 years in the Copenhagen CF Centre at Rigshospitalet. At the Copenhagen CF Centre, patients with chronic infections are treated for 14 days every third month

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with intravenously (IV) administered antibiotics. This treatment regimen has been shown to increase survival significantly [2]. Between the IV treatments, patients are given oral and/or inhaled antibiotics according to the antimicrobial susceptibility testing [3]. It is therefore important that the concentration of the antibiotic used reaches therapeutic levels to avoid development of resistance and treatment failure.

Numerous antimicrobials are either substrates or inhibitors of the hepatic enzyme subfamily cytochrome P4503A4 (CYP3A4) [4]. Thus, CF patients receiving concomitantly administered antimicrobial agents are at risk of kinetic drug interactions and subsequent adverse effects [5,6]. This leads to significant difficulties when trying to achieve optimal treatment dosage.

Clarithromycin (CLR) is frequently used in the treatment of CF patients. It is extensively metabolised by CYP3A to 14-hydroxyCLR [7] and exerts inhibitory properties. Traditionally, the treatment dosage is calculated based on body weight and renal function of the patient. Inter-individual differences in the pharmacokinetics of administered drugs are significant contributors to the variability of both the treatment effect and severity of side effects [8]. It is therefore important to have reliable methods, which may predict the pharmacokinetics of CLR to achieve the optimal treatment dosage.

Among the human P450 enzymes, CYP3A is the most abundant P450 isoenzyme, and approximately 37% of all currently used drugs are substrates for CYP3A [9]. There is a wide inter-individual variation in the CYP3A activity (up to 40–100 folds) [10].

Four genes (*CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43*) encoding for the CYP3A enzymes have so far been described [11] with *CYP3A4* and *CYP3A5* being regarded as the most important [12]. Several Single Nucleotide polymorphisms (SNPs) in the *CYP3A* genes have been described but their importance have yet to be clearly established [13]. Active CYP3A5 enzyme expression has been found in only 10–30% of Caucasians and 50–60% of African Americans [14]. In these individuals CYP3A5 accounts for up to 50% of the metabolism of a drug and may play a significant role in the metabolism of specific drugs. While CYP3A7 is active only during the foetal life stage and its expression is down-regulated shortly after delivery, mRNA expression has been demonstrated in 11% of an adult population [12]. CYP3A43 expression is distributed ubiquitously in the body, but with the highest expression in the prostate gland and in the testes [11] however only contributes to 0.1–5% of CYP3A mRNA compared to CYP3A4 [15].

The Erythromycin Breath Test (ERMBT) uses the unique N-demethylation of erythromycin by CYP3A, and has gained acceptance as an in vivo assay of hepatic CYP3A activity measurement [16], and may be used to predict blood levels of drugs metabolised by CYP3A [17].

The objectives of this study were to investigate the relationships between ERMBT, *CYP3A* genotype and CLR pharmacokinetics with the perspective of improving the treatment with antibiotics in a very vulnerable group of patients.

2. Material and methods

2.1. Patients

Clinically stable CF-patients with a chronic pulmonary infection aged ≥ 18 years were eligible for the study. Patients were excluded if they had allergy to CLR and erythromycin. Any treatment with ciprofloxacin, azithromycin, voriconazole or other pharmaceuticals that might influence CYP3A activity was discontinued at least 14 days before ERMBT was performed. The patients were asked not to consume grapefruit or grapefruit juice in the same period.

2.2. The Erythromycin Breath Test (ERMBT)

ERMBT was performed in each patient 1 h before administration of CLR. The ERMBT was performed essentially as described by Watkins et al. [17]. Patients were given 0.15 MBq of [^{14}C-N-methyl] erythromycin in 1 mL of ethanol (Isotope drugstore, USA) intravenously as a bolus with 5 mL of 5% dextrose, and breath samples were collected at 0, 3, 10, 20, 30, 40, 50, and 60 min after injection. The samples were collected by the patients breathing into a collection tube through a 1-meter long catheter. A solution consisting of 4 mL of hyamine hydroxide (Canberra Packard, Groningen, The Netherlands) and ethanol 1:1 with a trace amount of thymolphthalein as an indicator was used for the trapping of 2 mmol of CO_2. The specific activity of ^{14}C was determined by scintillation counting (scintillator, Perkin Elmer, USA). The ERMBT results were expressed as the calculated percentage of administered ^{14}C exhaled during the first hour after injection assuming an endogenous production of 5 mmol CO_2 per m^2 body surface area per minute [4].

2.3. Clarithromycin

After the ERMBT each patient received one tablet of 500 mg CLR (Clarithromycin Hexal 500 mg). Immediately before CLR intake, a blood sample was taken (t = 0). Additional blood samples were taken at 15, 45, 60, 90,120, 150 and 180 min and at 6 and 12 h respectively, in 2 mL EDTA tubes. The samples were centrifuged and plasma was separated and stored at −80 °C.

2.4. CYP3A4 genotyping

Five SNPs in *CYP3A4* were assessed by PCR and direct sequencing using primes (CYP3A4*1B; c.104-288G > A, CYP 3A4*3; c.1334T > C, CYP3A4*17; c.566T > C, CYP3A4*2; c.664T > C and CYP3A4*18A, c.878T > C), and PCR conditions as previously described by He et al. 2005 and Sata et al. 2000 [15,18]. PCR products were purified using NucleoFast96PCR plate (Machery–Nagel), directly sequenced on the forward strand using BigDyeTerminator V1.1, and subsequently resolved on an ABI3130xl (Applied Biosystems, Foster City, USA). NM_017460 was used as reference sequence.

2.5. CYP3A5 genotyping

Seven SNPs in the CYP3A5 gene were detected using a multiplex PCR assay followed by a multiplex single base extension (SBE) reaction. The assay includes six primer pairs that simultaneously amplify fragments of the *CYP3A5* gene. The primer design was performed using Primer 3.0 v. 0.2 [19] and OligoAnalyzer v. 1.0.3 (Freeware; TeemuKuulasmaa, Finland). RP-HPLC purified primers and probes were purchased from DNA-technology A/S (Arhus, Denmark). Table 1 shows the primer sequences.

The multiplex PCR was amplified in a 25 μL reaction containing 1 μL template DNA, 1× buffer (Amplitaq gold buffer, Applied Biosystems), 5.5 mM $MgCl_2$, 160 μM of each dNTP, 0.04 to 0.32 μM of each primer (Table 1) and 1 Unit Amplitaq gold DNA polymerase (Applied Biosystems). The amplification was done in a GeneAmp® PCR System 9700 (Applied Biosystems) with the following programme: Initial denaturation at 94 °C for 10 min followed by 30 cycles with 94 °C for 30 s, 60 °C for 30 s and 72 °C for 60 s. Final extension at 72 °C for 10 min.

2.6. Single base extension (SBE)

A multiplex SBE reaction was developed in order to simultaneously detect the seven investigated SNPs. SBE primers were designed using the programme OligoAnalyzer v. 1.0.3 (Freeware; TeemuKuulasmaa, Finland). The lengths of the template specific parts of the primers were between 20 and 25 bps. In order to distinguish between the signals from the SBE primers, the lengths were adjusted by the addition of pieces of 'neutral' sequences at the 5′-end of the primers (Table 2). These sequences did not match any human sequence in the NCBI database [20]. The purification of the PCR products, minisequencing and subsequent purification of the minisequencing reaction were carried out in accordance to the SNaPshot protocol of the manufacturer (Applied Biosystems). One microlitre of the purified SBE reaction was analysed on an AB Prism 3130 Genetic Analyser with a 36 cm capillary array, POP-4 polymer and 10 s at 3000 V injections. GeneScan-120 LIZ™ was used as internal size standard. The data were analysed using GeneScan Analysis software v. 3.7 (Applied Biosystems). All samples were tested in duplicates.

2.7. CLR and 14-hydroxyCLR concentration measurement

Plasma-concentrations of CLR and 14-hydroxyCLR were determined in the Laboratory for Clinical Toxicology and Drugs Analysis of the Department of Hospital and Clinical Pharmacy at the University Medical Center in Groningen using a validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) [21]. CLR and 14-hydroxyCLR were measured by protein precipitation, followed by LC with tandem mass detection. Of each plasma sample 10 μL was mixed with 750 μL precipitation reagent (methanol and acetonitrile 4:21 v/v) containing the internal standard and were vortexed for 1 min, stored at −20 °C for about 30 min to promote protein precipitation and thereafter centrifuged at 11,000 x*g* for 5 min. From the clear upper layer 5 μL was injected on a 50 mm × 2.1 mm C18, 5-μm analytic column (HyPurity Aquastar, Interscience Breda, The Netherlands) for chromatographic separation. The column temperature was set at 20 °C. The mobile phase had a flow of 0.3 mL/min and consisted of an aqueous buffer (containing ammonium acetate 10 g/L, acetic acid 35 mg/L and trifluoroacetic anhydride 2 mL/L of water), water and acetonitrile. The detector was operating in electrospray positive ionization mode and performed selected reaction monitoring (SRM) as scanning mode. The mass parameters of m/z 748.5–590.2 (collision energy 18 eV) and m/z 764.4–606.2 (collision energy 20 eV) were measured with scan width of 0.5 m/z for CLR and 14-hydroxyCLR. The ion source spray voltage was set at 3500 V, the sheath and auxiliary gas pressure at 35 Arbitrary Units (AU) and 5 AU, respectively and the capillary temperature at 350 °C. The recoveries ranged from 100.1% to 116.9%, depending on the concentration. The accuracy was between 94.6% and 100.2% for CLR and between 90.5 and 105.0% for 14-hydroxyCLR, depending on the concentration level. The intra- and interassay coefficients of variation were less than 7.7% over the ranges of 0.1 to 10 mg/L for both CLR and 14-hydroxyCLR. The lower limit of quantitation was 0.1 mg/L for both CLR and 14-hydroxyCLR. CLR-containing samples were stable (<10% loss) for at least 12 months at −20 °C.

2.8. Pharmacokinetics analysis

Pharmacokinetic parameters (Cmax, Tmax and AUC) were determined by the use of GraphPad Prism 4. AUC was calculated using the trapezoidal rule from time = 0 to last time-point.

Table 1
Sequences of the primer sets used for CYP3A5 genotyping.

SNP	Forward primer (5′-3′)	Reverse primer (5`-3`)	μM	Amplicon size (bp)
CYP3A5*3	TTATGGAGAGTGGCATAGGAG	AGTTGTACGACACACAGCAAC	0.06	226
CYP3A5*4, *6	TACAGCATGGATGTGATTACTG	TGAGAGAAATAATGGATCTAAGAAAC	0.08	132
CYP3A5*5	TGTTGAACTCTAGTCTTTAGGC	GTCCCCAGATTCATTCTTTACA	0.04	177
CYP3A5*7	TACTGCATGGACTCAGTTGAG	ACTCCATCTGTACCACGGC	0.06	105
CYP3A5*8	AGTAACTCACCAGCCCTCTG	GCCCTGGAATTCCCAGTC	0.32	114
CYP3A5*9	TGTCTGATCTGGAGCTCGCA	TCCCTTCATCTCCAGGGGT	0.08	187

Table 2
Neutral sequences and the target specific sequences used for the single base extension assay.

SNP	Neutral sequence (5′-3′)	Target specific sequence (5′-3′)	μM	Primer size (bp)
CYP3A5*3	ACAA	GGTCCAAACAGGGAAGAGATA	0.15	25
CYP3A5*4	GTCTGACAA	TGCTCTCCACAAAGGGGTCT	0.06	29
CYP3A5*5	AAAGTCTGACAA	CAGCGGAAAACTCAAGGAGG	0.15	32
CYP3A5*6	AAAGTCTGACAA	CTAAGAAACCAAATTTTAGGAACTT	0.1	37
CYP3A5*7	CACGTCGTGAAAGTCTGACAA	TTTCCTTCCAGGCACCACCT	0.04	41
CYP3A5*8	CACGTCGTGAAAGTCTGACAA	GTCTCTTAAAAAGTCCATGTGTAC	0.04	45
CYP3A5*9	TGCCACGTCGTGAAAGTCTGACAA	CAGAAACTGCAAAAGGAGATTGAT	0.04	48

2.9. Ethics

The study was approved by the Ethics Committee of Copenhagen (J.no (KF (01) 261286)). Written informed consents were obtained from all patients.

3. Results

3.1. Patients

Twenty-one CF patients (19 males and 3 females) were included in the study from February 2006 to April 2010. The median (range) age of the patients was 33 years (21–53 years). Sixteen patients were chronically infected with *Pseudomonas aeruginosa* and one with *P. aeruginosa* and *Stenotrophomonas maltophilia*. Two patients were chronically infected with *Achromobacter xylosoxidans* and two with *Burkholderia cepacia* complex. The lung function was evaluated in each patient by FEV1% median (range) at 67 (30–96). Further demographic data are shown in Table 3.

Table 3
Demographic data from the 21 CF patients included in the study.

	Patients N = 21
Genotype:	
Δ508 homozygotes	16
Δ508 heterozygotes	5
Class 2	19
Other than 2	2
Diabetes	
Yes (no)	7 (14)
FEV1% of predicted median (range)	65 (30 to 96)
Chronic infection	
P. aeruginosa	16
P. aeruginosa and *S. maltophilia*	1
A. xylosoxidans	2
B. cepacia complex	2
Median age (range)	34 (21 to 53)
Gender, male (female)	18 (3)
Carbamid (mmol/L) median (range)	7.2 (4.1 to 12.1)
Creatinine (μmol/L) median (range)	75.5 (55 to 126)
BMI (kg/m^2) median (range)	22.4 (16.3 to 27.1)
ALAT (U/L) median (range)	31 (13 to 211)
CRP (mg/L) median (range)	5 (0 to 17)

Demographic data of the patients included in the study.

3.2. Pharmacokinetics of CLR

A 10-fold variation in AUC for CLR, median (range) 881 μg/mL × min (274–2831) and a 12-fold variation in AUC for 14-hydroxyCLR median (range) 366 μg/mL × min (84–1041) were observed. Variation in Cmax was 16-fold for CLR median (range) 3.4 μg/mL (0.5–7.8) and 11-fold for 14-hydroxyCLR median (range) 0.9 μg/mL (0.2–2.2). The means are shown in Table 4. The median (range) Tmax was 96 min (34–360) and a median (range) T ½ at 437 min (321–2575 min) for CLR and a median (range) Tmax at 123 min (45–360 min), and a median (range) T ½ at 768.5 min (415–1848) for the 14-hydroxyCLR. The ratio between the CLR and its active metabolite 14-hydroxyCLR showed a large variability with a median (range) ratio of 2.4 (1.2 to 8.9). Two patients (numbers 1 and 7) had very high ratio at 8.9 and 8.7 respectively, which is almost 4 times above the median.

3.3. ERMBT

A large variation in the CYP3A activity expressed as (ERMBT %^{14}C/h) values was found with an 8-fold variation and a median (range) of 0.8 (0.25–2.0). One patient (number 20) had a very high activity at 2%^{14}C/h, 2.5 fold higher compared to the median. Two patients (numbers 1 and 7) had very low activities (0.25 and 0.4%^{14}C/h, respectively), which are 3 and 2 folds below the median. Neither patient number 1, 7 or 20 had a different genotype compared to the rest of the population that could explain this difference, they were all homozygotes for *CYP3A4*1A*, and *CYP3A5*3*.

3.4. Correlation between the ERMBT and CLR metabolism

A significant correlation between the metabolism of CLR, expressed as the AUC CLR/AUC 14-hydroxyCLR ratio and the CYP3A4 activity expressed as the ERMBT was observed (Fig. 1), ($P = 0.0092$; $r^2 = 0.31$). No correlation between the plasma-concentration of CLR and hydroxyCLR and the ERMBT was found.

3.5. CYP3A4/5 SNPs

No differences in *CYP3A4* genotypes between patients were found. Two patients (patient numbers 4 and 9) were found to have one copy of a functional *CYP3A5* gene (*CYP3A5*1*) (9.5% of the population) and none were homozygote *CYP3A5*1*. There

Table 4
Pharmacokinetics of CLR and its active metabolite 14-hydroxy-CLR is shown.

	AUC (μg/mL × min) (mean+/−SD)	Cmax (μg/mL) (mean+/−SD)	Tmax (min) (mean+/−SD)	T ½ (min) (mean+/−SD)
Clarithromycin	1082+/−615	3.6+/−2	124+/−85	582+/−463
14-hydroxy-CLR	425+/−233	1.0+/−0.5	135+/−87	845+/−357

The pharmacokinetics of CLR and its active metabolite 14-hydroxy-CLR.

was no obvious correlation between the SNP genotypes and CYP3A4 activity. The two patients with one copy of the functional *CYP3A5* gene (*CYP3A5**1) did not show higher CYP3A activity (0.98 and 0.83, respectively) (ERMBT %^{14}C/h), or difference in the (AUC CLR/AUC 14-hydroxyCLR) ratio (2.23 and 1.75, respectively).

4. Discussion

We observed a large variability in the pharmacokinetic parameters of CLR and its active metabolite in the 21 CF patients after administration of a single dose of 500 mg CLR. AUC varied 10-fold and Cmax 16-fold.

Several factors can influence whether therapeutic drug concentration is reached, and it is well known that different pharmacokinetic parameters are altered in CF patients [22] making antibiotic dosage difficult. Gilljam et al. found sub-therapeutically serum level of CLR in ⅔ of the patients 2 h after oral intake [23] stressing the need of therapeutic drug monitoring. This corresponds to our results, which suggests that some patients might be at risk of toxic side effects and others of treatment failure.

We found an 8-fold variation in the CYP3A activity, 2 patients with very low values (numbers 1 and 7), and 1 patient (number 20) with very high values compared to the median. Giving the former two patients with low CYP activity a therapeutic CLR dose may lead to toxic high serum-concentrations while the same dose may result in therapeutic failure in patient number 20 with a high CYP activity.

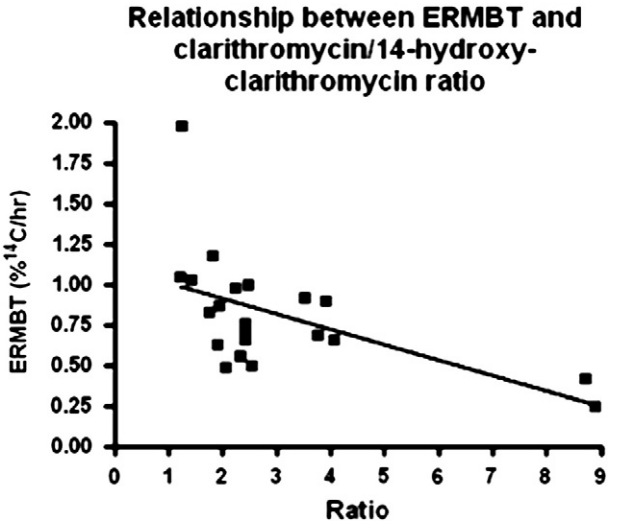

Fig. 1. Correlation between the metabolism of CLR, expressed as the AUC CLR/AUC 14-hydroxy-CLR ratio, and the CYP3A4 activity expressed as the ERMBT (P = 0.0092; r^2 = 0.31). Each dot represents a patient.

We therefore investigated if there was a correlation between the ERMBT and the metabolism of CLR expressed as the ratio between the AUC CLR/AUC 14-hydroxyCLR since the macrolide antibiotic clarithromycin is extensively metabolised by CYP3A to 14-hydroxyCLR [7]. The ratio of clarithromycin to14-hydroxyCLR (e.g. the ratio of parent compound to metabolite area under the curve(s)) may therefore relate to enzyme activity. A high ratio corresponding to low enzyme activity, and the opposite with a low ratio. We found a significant correlation between the CLR/metabolite ratio and the ERMBT value. This indicates that patients with high ERMBT values metabolise CLR at a greater rate compared to patients with low ERMBT values. The ERMBT is a validated method of assessing the CYP3A activity in vivo although its capability of predicting total drug clearance is less clear [4,17]. Schmidt et al. found that the ERMBT could be used to predict cyclosporine and tacrolimus nephrotoxicity after liver transplantation [16], and patients with low ERMBT values are more likely to have poor drug clearance [4]. As the ERMBT is easy to perform and fast to analyse, it would be interesting to see if the use of this test to individualize treatment could result in optimized treatment with subsequent better results.

One of the limitations in the study is that the ERMBT is performed by IV administered erythromycin, and CLR is given orally thus, the results may be influenced by the difference in bioavailability. The bioavailability of 250 mg CLR is described to be 52–55% in healthy volunteers [24]. However, this may be different in CF patients due to pancreatic insufficiency, bile acid malabsorption and gastric acid hypersecretion making it difficult to estimate bioavailability and the role of the intestine in these patients. Beringer et al. found no difference of the bioavailability of azithromycin in CF patients compared to healthy controls [25] but Han et al. found that CF patients had lower bioavailability of voriconazole compared to controls [26]. It is therefore difficult to predict the contributing role of the intestines in the metabolism of CLR, which would be interesting to investigate. We are well aware that our patient group is small, and larger studies will be required to confirm the conclusions of the study. Furthermore, we were not able to investigate the potential influence of renal clearance of CLR, which might have an influence on the results as well, and in future studies we would like to take this into account. More males than females were included in our study, due to the fact that we were not able to recruit more females among our adult CF patients. This is only partly due to the general gender distribution in adult CF populations since males have better prognosis than females [27].

The ERMBT test may be used as a clinical tool to predict the metabolism of CLR in CF patients. Our results indicate that CF patients could benefit from a pre-treatment ERMBT in order to identify individual patients in which dose titration might be useful in order to avoid toxicity or therapeutic failure.

To investigate if the activity correlated with different CYP3A genotypes we assessed five different SNPs in the *CYP3A4* gene (*CYP3A4*1B*, *CYP3A4*3*, *CYP3A4*17*, *CYP3A4*2* and *CYP3A4*18A*). The CYP3A4*1B genotype is very common among African Americans (35–67%), present in 2–9.6% of Caucasians but is totally absent in the Asian population [28]. The effect of this SNP is controversial and has been associated with high enzyme activity in some studies and with low enzyme activity in others [29]. In Caucasians, the prevalence of *CYP3A4*2* has been reported to be 2.7%, the prevalence of *CYP3A4*3* to be 0.47–4%, the prevalence of *CYP3A4*17* to be 2.1% and the prevalence of *CYP3A4*18A* to be 0 [28]. All the patients in this study were genotyped to be homozygote for the wild type *CYP3A4* allele (homozygote for *CYP3A4*1A*). The assessed *CYP3A4* SNP's all display a low minor allele frequency in Caucasians, and thus, our results are not unexpected, but the low number of tested patients do not allow a firm conclusion. The direct sequencing approach did not reveal other variations. There was no obvious genotype–phenotype correlation.

We genotyped 7 SNPs in the *CYP3A5* gene and these are shown in Table 2. CYP3A5*3, CYP3A5*6 and CYP3A5*7 are associated with decreased activity, where the latter two are only found in an African, or African American population [29]. CYP3A5*3 is very common in Caucasians with 83% being CYP3A5*3 homozygote and approx. 17% being heterozygote [30]. Expressing of the CYP3A5 enzyme has been associated with a greater clearance of cyclosporine and tacrolimus [31] in patients.

All but 2 (9.5%) of our patients were genotyped as homozygotes for the CYP3A5*3 allele (*CYP3A5*1/*3*). Therefore the majority of the patients, in accordance with the literature, have no functional *CYP3A5* gene. Due to the homogeneity of our cohort, i.e. the high prevalence of the CYP3A5*3 polymorphism, we were not able to detect a genotype–phenotype correlation. A larger cohort of CF patients will be required to evaluate this correlation.

The assessed CYP3A polymorphisms could not explain the large variability in the pharmacokinetics of CLR, and other factors e.g. the possible differences of the bio-availability in CF patients, or other CYP enzymes might explain some of the variability, but this would need further investigations.

In conclusion, we found a high degree of variation in the pharmacokinetic parameters of CLR and its metabolite. This may lead to treatment failure or adverse drug effects. There was a significant correlation between the metabolism of CLR and the ERMBT. Further studies are therefore needed to evaluate the value of the ERMBT as a method to individualize the dosage of CLR or other drugs in CF and other patient groups as a supplement to conventional parameters like body weight or renal function.

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C. S. D: Nothing to declare.

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